*Trans*NGS® Library Quantification Kit for Illumina®

Cat. No. KQ101
Storage: at -20°C in dark for one year

Description

*Trans*NGS® Library Quantification Kit for Illumina® provides all reagents needed for qPCR based quantification of DNA libraries prepared for Illumina next generation sequencing platforms. Five linear DNA Standards with different GC contents are provided for customers to choose from. The kit also contains PCR primers which target the P5 and P7 adaptor sequences and *Trans*NGS® Library Quantification qPCR SuperMix (2×) for library amplifications.

Highlights

Five sets of linear DNA Standards (25%, 37.5%, 50%, 62.5%, and 75% of GC contents) to choose from Each DNA Standard contains six 10-fold dilution series of a linear 420 bp template *Trans*NGS® Library Quantification qPCR SuperMix (2×) enables accurate library amplification with various GC contents.

Application

Quantification of DNA libraries prepared for Illumina next generation sequencing platforms with P5 and P7 adaptor sequences.

Kit Contents

Component	KQ101-01 (100 rxns)	KQ101-02 (500 rxns)
*Trans*NGS® Library Quantification qPCR SuperMix (2×)	1 ml	5×1 ml
Library Quantification Primer Mix (20×)	100 µl	500 µl
DNA Standards (S1-S6)	24 µl each	120 µl each
Library Dilution Buffer (10×)	1 ml	5 ml
Passive Reference Dye (50×)	40 µl	200 µl
Nuclease-free Water	10 ml	50 ml

a) Five sets of linear DNA Standards to choose from. The kit comes with DNA Standard with 50% of GC contents if customers not make the choice.
b) Six concentrations (S1-S6) are supplied for each DNA Standard.

Table 1 DNA Standard Concentration Copy Number

DNA Standard	S1	S2	S3	S4	S5	S6
Molarity (pM)	20	2	0.2	0.02	0.002	0.0002
Copy Number (copies/µl)	12×10^6	12×10^5	12×10^4	12×10^3	12×10^2	12×10^1

Procedures

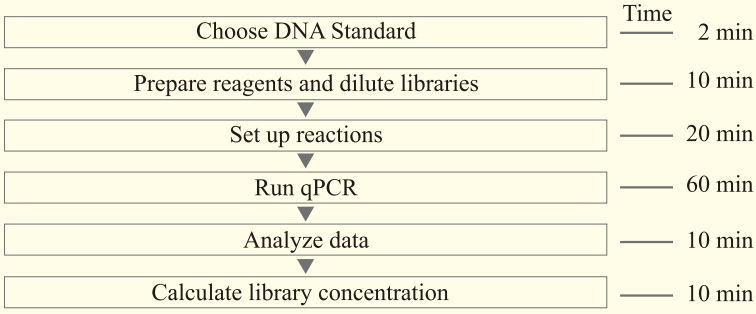

Figure 1 qPCR quantitative workflow of NGS libraries

Choose DNA Standard

Use the following guides to choose DNA Standard

Library GC Contents	DNA Standard to Use
18%-31%	25% GC DNA Standards (S1-S6)
31%-42%	37.5% GC DNA Standards (S1-S6)
42%-56 %	50% GC DNA Standards (S1-S6)
56%-68%	62.5% GC DNA Standards (S1-S6)
68%-80%	75% GC DNA Standards (S1-S6)

Note: For library with unknown GC contents, check the GC contents of the related species (see the Appendix).

1. Prepare reagents and dilute libraries

Thaw kit components, mix well and centrifuge briefly. Place reagents on ice.

Prepare the 1×Library Dilution Buffer by making a 1:10 dilution of the 10× Library Dilution Buffer with Nuclease-free Water. Prepare sufficient buffer. Each library needs about 300 µl of the buffer. The 1× Library Dilution Buffer can be stored at 2-8°C for upto 1 week. Measure library concentration using Agilent 2100 Bioanalyzer. Dilute all libraries with 1× Library Dilution Buffer. Make sure the concentration of the diluted library is in the same range of the DNA Standard. We suggest adding 1 µl of library sample to 999 µl of the 1×Library Dilution Buffer to create a 1:1,000 dilution. We usually use 1:10,000 and 1:20,000 dilutions for qPCR analysis. The 1:10,000 dilution can be prepared by adding 10 µl of the 1:1,000 dilution to 90 µl of the 1× Library Dilution Buffer.

Note: Do not dilute library with water, TE or other buffers.

2. Prepare qPCR Assays

For best results, we recommend running each DNA Standard and library sample in triplicate.

Prepare the required volume of Master Mix, Primer Mix, DNA (DNA Standard, or diluted library sample, or the 1× Library Dilution Buffer as negative control) as recommended below. Use the right Reference Dye according to the qPCR instruments.

Table 3 qPCR reaction setup (20 µl)

Component	Volume
*Trans*NGS® Library Quantification qPCR SuperMix (2×)	10 µl
Primer Mix (20×)	1 µl
Passive Reference Dye I / II (optional)	-/0.4 µl
Nuclease-free Water	5 or 4.6 µl
DNA Standard/Library	4 µl
Total	20 µl

3. Run qPCR

qPCR cycling can be performed with fast mode (two-steps) or standard mode (three-steps). An optional denaturation/melt cure can be included if desired.

Two-step qPCR

95℃ 5 min
95℃ 25 sec
60℃ 45 sec
35 cycles
Dissociation Stage

Three-step qPCR

95℃ 5 min
95℃ 20 sec
60℃ 20 sec
72℃ 30 sec
35 cycles
Dissociation Stage

Notes

a) Please select the "EvaGreen" or "SYBR Green" channel to collect signal.

b) Two-step qPCR is recommended for library with medium GC contents and the library size is less than 700 bp.

c) For library with high or low GC contents, it is recommended to use three-step qPCR.

d) For library with an average length longer than 700 bp, we suggest to use three-step method and increase the extension time to 50 seconds.

e) This kit is not optimized for library larger than 1 kb.

4. Analyze data

Replicate data points should differ by ≤0.3 cycles. If the data set contains many outliers, results are unlikely to be reliable. In that case, it is recommended to repeat the assay. Use the standard curve to convert the average Cq score for each dilution of every library that was assayed to average concentration (in pM). The standard ΔCt between two consecutive dilution points should be between 3.1-3.6. The Ct value between the DNA Standard and the negative control should be greater than 3. The standard curve correlation coefficient R^2 should be no less than 0.99, and the slope should be between -3.1 ~ -3.6, indicating that the amplification efficiency is between 90% ~ 110%. Use table 4 to calculate the library concentration.

Notes

a) Use at least 4 dilution points to generate the standard curve. If the Ct number from NTC > the Ct number from (S6) + 3, all six dilution points from the DNA Standard can be used to generate the standard curve.

b) If the Ct number from (S6) + 3 is larger than the Ct number from NTC, and Ct (S6) - Ct (S5) is between 3.1-3.6, only use the Ct numbers from S1-S5 to generate the standard curve.

c) If the Ct number from S5 + 3 > the Ct number from NTC, all data is not reliable and the experiment needs to be repeated.

d) It is recommended to repeat the experiment with different library sample dilutions if the Ct value of the library falls outside of the standard curve.

Table 4 Concentration of DNA Standard

DNA Standard	S1	S2	S3	S4	S5	S6
Concentration (pM)	20	2	0.2	0.02	0.002	0.0002
Log[pM]	Log[20]	Log[2]	Log[0.2]	Log[0.02]	Log[0.002]	Log[0.0002]

5. Calculate library concentration

The concentration of the diluted Library (pM) = [420 bp / library average length (bp)]× the diluted Library initial concentration from the measurement (pM).

Concentration of original library (nM) = the concentration of the dilution library obtained from above (pM) × dilution folds /1,000.

Notes: It is recommended to use 2 dilution points and use the average to obtain the original library concentration.

Examples

Two DNA libraries were prepared from 50 ng of *Arabidopsis thaliana* genomic DNA and 50 ng of *Escherichia coli* genomic DNA using *Trans*NGS® Tn5 DNA Library Prep Kit for Illumina®. The average fragment length and concentration of these 2 libraries were measured with Agilent Bioanalyzer (Table 5).

An initial 1/10,000 dilution and one additional 2-fold (i.e. 1/20,000) dilution of these two libraries was prepared. Because the GC content of genomic DNA of *Arabidopsis thaliana* is about 36%, DNA Standard with 37.5% of GC contents was used to measure the library concentration. And the GC content of *Escherichia coli* is about 50%, DNA Standard with 50% of GC contents was used for *Escherichia coli* library. The results and the calculated data are shown in table 5.

Table 5

	Library 1		Library 2	
Average fragment length (Bioanalyzer)	490 bp		560 bp	
Estimated concentration (Bioanalyzer)	6.95 ng/μl = 21.52 nM		7.62 ng/μl = 22.56 nM	
Dilutions for qPCR	1/20,000	1/10,000	1/20,000	1/10,000
GC content of library	about 36%		about 50%	

The DNA Standard	DNA Standard with 37.5% of GC		DNA Standard with 50% of GC	
Triplicate Cq scores	11.91	10.82	11.11	10.21
	11.94	10.93	11.21	10.13
	11.86	10.86	11.25	10.28
Average Cq score	11.90	10.88	11.19	10.21
ΔCt	1.02		0.98	
Average concentration based on the standard curve (pM)	1.102	2.277	1.375	2.685
Average size-adjusted concentration for diluted library (pM)	0.9445	1.952	1.0315	2.014
Average final calculated concentration of undiluted library (nM)	18.89	19.52	20.63	20.14
Deviation between final concentrations calculated from different dilutions	4.56%		2.43%	
Original Library Concentration	19.21 nM		20.38 nM	

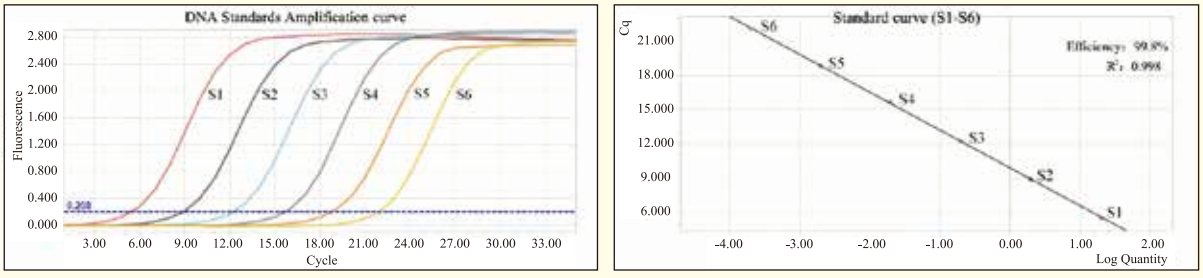

Figure 2 Amplification curve (upper) and the standard curve (S1-S6)

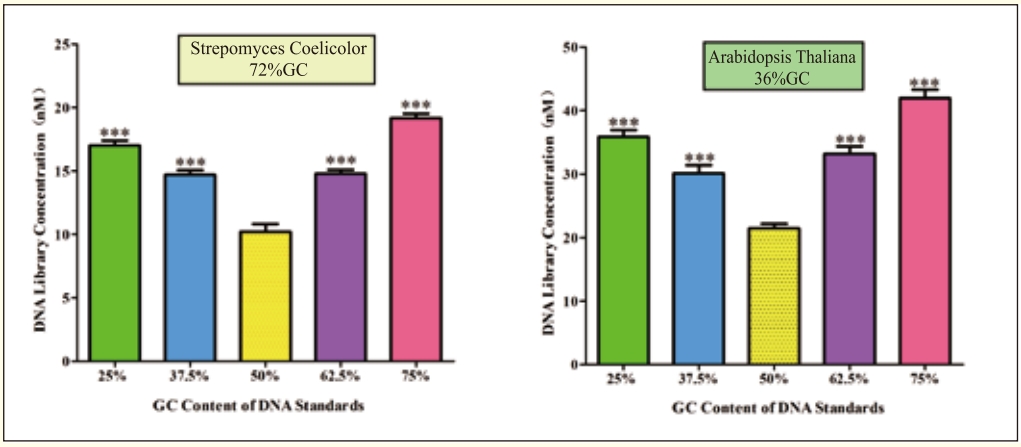

Figure 3 Library Concentration measured with different DNA Standards.
Left: *Streptomyces coelicolor*; right: *Arabidopsis thaliana*

Appendix - Reference information

GC contents of the common species			
Name	GC Content (%)	Genome Size（Mb）	Organism
Homo sapiens	41.1	2996.42	Animals
Mus musculus	42.6	2671.82	Animals
Rattus norvegicus	42.3	2870.18	Animals
Oryctolagus cuniculus	44.1	2737.46	Animals
Sus scrofa	42.5	2808.53	Animals
Drosophila simulans	43.4	121.27	Animals
Caenorhabditis elegans	35.5	100.73	Animals
Capra hircus	42.7	2779.32	Animals
Parus major	41.4	1020.31	Animals
Esox lucius	42.2	904.45	Animals
Taenia saginata	42.5	168.93	Animals
Plasmodium falciparum	22.9	23.37	Animals
Arabidopsis thaliana	36.1	119.67	Plants
Solanum lycopersicum	34.8	760.07	Plants
Solanum tuberosum	35.6	705.93	Plants
Oryza sativa	43.1	359.94	Plants
Triticum aestivum	44.8	1.27	Plants
Zea mays	46.8	2222.33	Plants
Nicotiana tabacum	39.2	3732.64	Plants
Brassica napus	37.4	930.51	Plants
Streptomyces coelicolor	71.9	9.05	Bacteria
Thermus thermophilus	69.4	2.15	Bacteria
Rhodopseudomonas palustris	64.9	5.44	Bacteria
Sphingobium xenophagum	62.9	4.49	Bacteria
Escherichia coli	50.6	5.44	Bacteria
Staphylococcus aureus	32.8	2.86	Bacteria
Saccharomyces cerevisiae	38.5	12.13	Fungi
Human immunodeficiency virus	42.1	0.00918	Viruses
Hepatitis B virus	48.5	3.18	Viruses
Bacillus phage Page	40.7	0.03987	Viruses

Recommended layout for 96 well plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC	NTC	NTC	Library 1	Library 1	Library 1	Library 5	Library 5	Library 5	Library 9	Library 9	Library 9
				1:10,000	1:10,000	1:10,000	1:10,000	1:10,000	1:10,000	1:10,000	1:10,000	1:10,000
B	Standard 6	Standard 6	Standard 6	Library 1	Library 1	Library 1	Library 5	Library 5	Library 5	Library 9	Library 9	Library 9
	0.0002 pM	0.0002 pM	0.0002 pM	1:20,000	1:20,000	1:20,000	1:20,000	1:20,000	1:20,000	1:20,000	1:20,000	1:20,000
C	Standard 5	Standard 5	Standard 5	Library 2	Library 2	Library 2	Library 6	Library 6	Library 6	Library 10	Library 10	Library 10
	0.002 pM	0.002 pM	0.002 pM	1:10,000	1:10,000	1:10,000	1:10,000	1:10,000	1:10,000	1:10,000	1:10,000	1:10,000
D	Standard 4	Standard 4	Standard 4	Library 2	Library 2	Library 2	Library 6	Library 6	Library 6	Library 10	Library 10	Library 10
	0.02 pM	0.02 pM	0.02 pM	1:20,000	1:20,000	1:20,000	1:20,000	1:20,000	1:20,000	1:20,000	1:20,000	1:20,000
E	Standard 3	Standard 3	Standard 3	Library 3	Library 3	Library 3	Library 7	Library 7	Library 7	Library 11	Library 11	Library 11
	0.2 pM	0.2 pM	0.2 pM	1:10,000	1:10,000	1:10,000	1:10,000	1:10,000	1:10,000	1:10,000	1:10,000	1:10,000
F	Standard 2	Standard 2	Standard 2	Library 3	Library 3	Library 3	Library 7	Library 7	Library 7	Library 11	Library 11	Library 11
	2 pM	2 pM	2 pM	1:20,000	1:20,000	1:20,000	1:20,000	1:20,000	1:20,000	1:20,000	1:20,000	1:20,000
G	Standard 1	Standard 1	Standard 1	Library 4	Library 4	Library 4	Library 8	Library 8	Library 8	Library 12	Library 12	Library 12
	20 pM	20 pM	20 pM	1:10,000	1:10,000	1:10,000	1:10,000	1:10,000	1:10,000	1:10,000	1:10,000	1:10,000
H				Library 4	Library 4	Library 4	Library 8	Library 8	Library 8	Library 12	Library 12	Library 12
				1:20,000	1:20,000	1:20,000	1:20,000	1:20,000	1:20,000	1:20,000	1:20,000	1:20,000

Passive Reference Dye

- Passive Reference Dye I (50×)

  ABI Prism7000/7300/7700/7900, Eppendorf, ABI Step One, ABI Step One Plus
- Passive Reference Dye II (50×)

  ABI Prism7500, ABI Prism7500 Fast, ABI Q6, ABI Quant Studio 6/7 Flex, ABI ViiA 7, Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000
- No Passive Reference Dye

  Roche LightCycler480, Roche Light Cycler96, MJ Research Chromo4, Opticon (II), Takara TP800, Bio-Rad iCycler iQ, iQ5, Bio-Rad CFX96, Bio-Rad C1000 Thermal Cycler, Thermo Pikoreal 96, Corbett Rotor Gene 6000, Corbett Rotor Gene G, Corbett Rotor Gene Q

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